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Combined ETA/ETB receptor blockade of human peritoneal mesothelial cells inhibits collagen I RNA synthesis

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Combined ETA/ETB receptor blockade of human peritoneal mesothelial cells inhibits collagen I RNA synthesis.

Background. Peritoneal fibrosis is a serious complication of peritoneal dialysis; however, the mechanisms are poorly understood. We studied osmolarity and physical stress-induced effects on collagen I RNA synthesis in human peritoneal mesothelial cells (HPMCs) and focused on endothelin as a possible mediator.

Methods. HPMCs were grown in a medium containing either D-glucose or glycerol to analyze the impact of osmolarity on mesothelial endothelin-1 (ET-1) release and on collagen I RNA synthesis [reverse transcription-polymerase chain reaction (RT-PCR)]. A cellular model of nonlaminar fluid shear stress and cellular stretch was used to analyze the effects of physical forces. To neutralize the endothelin effects, a combined ETA/ETB receptor antagonist (LU 302 872) was choosen.

Results. Glucose, but not glycerol, increased mesothelial ET-1 release in a concentration and time-dependent manner ($P < 0.05$ vs. controls). Collagen I RNA synthesis was significantly higher in glucose-challenged cell cultures ($P < 0.05$ vs. controls). The glucose-mediated collagen I RNA synthesis was completely inhibited by adding the combined ETA/ETB receptor antagonist to the medium. Fluid shear stress, but not cellular stretch, led to a significant increase in the mesothelial ET-1 release ($P < 0.005$ vs. controls) and collagen I RNA synthesis ($P < 0.05$ vs. controls). LU 302 872 completely inhibited these effects.

Conclusion. We found that glucose and fluid shear stress are potent stimuli for ET-1 release and collagen I RNA synthesis in a model cellular system. Although our system is highly artificial, our findings raise the hypothesis that similar effects may occur in the peritoneal membranes of peritoneal dialysis patients and suggest that endothelin might be involved.

Key words: peritoneal dialysis, peritoneal fibrosis, osmolarity, physical forces, endothelin, endothelin receptor antagonist.

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In long-term peritoneal dialysis, chronic scarring of the peritoneal membrane may result in treatment failure [1]. The mechanisms involved in the initiation and progression of peritoneal fibrosis are not well understood. The dialysate is not a physiologic fluid and may play an important role. The high glucose concentrations may alter mesothelial cell function [2–4] and trigger the release of profibrotic mediators [5–7]. Alternatively, the high osmolarity could play a role [8, 9]. Multiple exchanges must be performed throughout the day with stretching and distension of the mesothelial cells. Conceivably, mechanical forces could be involved. Such forces are important in regulating cell proliferation and differentiation in various cells including endothelial cells [10, 11], vascular smooth muscle cells [12], cardiac myocytes [13], and pulmonary mesothelial cells [14]. We recently reported on the effects of dwell volume and osmolarity of peritoneal dialysate on peritoneal endothelin-1 (ET-1) release in stable peritoneal dialysis patients [15]. We found that increasing dwell volumes from 1500 mL to 2500 mL per dwell led to a significant increase in peritoneal ET-1 release. Furthermore, changing the dialysate glucose concentration from 1.36% to 3.86% activated the peritoneal ET-system. Since endothelin is known to exert substantial profibrotic properties [16, 17], we reasoned that volume and osmolarity-induced ET-1 release might play a role in the process of fibrotic remodeling. To test this notion further, we have conducted a series of experiments in cultured HPMCs.

METHODS

HPMC isolation

We obtained intraoperative specimens of omentum from patients who had given written informed consent (IRB Charité). The pieces (3 to 5 cm^2) were washed three times with sterile phosphate-buffered saline (PBS), pH 7.3 (PAA Laboratories GmbH, Linz, Austria), and then

incubated with rotation in 0.25% (wt/vol) trypsin-0.01% (wt/vol) ethylenediaminetetraacetate (EDTA) solution (Sigma Chemical Co., St. Louis, MO, USA) for 20 minutes at 37°C. The tissue was then removed and trypsin solution containing free mesothelial cells was centrifuged at 411 × *g* for 10 minutes at 4°C. The supernatant was discarded. The cell pellet was washed once with PBS and then suspended in minimum essential medium D-valin (Life Technologies, Grand Island, NY, USA) supplemented with 10% fetal calf serum (FCS) (vol/vol) (Biochrom KG, Berlin, Germany), 100 U/mL penicillin (Biochrom KG), 100 μg/mL streptomycin (Biochrom KG), and 2 mmol/L L-glutamine (Biochrom KG). After seeding into uncoated 75 cm^2 tissue culture flasks, the cells were maintained in the same medium and incubated at 37°C under an atmosphere of 5% CO_2/95% air in a humidified incubator. The medium was replaced every 2 to 3 days. HPMCs from the second passage were used for the experiments. Confluent cells were polygonal and formed a regular monolayer in light microscopy. HPMCs were characterized by the alkaline phosphatase-antialkaline phosphatase (APAAP) technique. For experiments with endothelin antagonist, we used a combined ETA/ETB receptor antagonist (LU 302 872) in a concentration of 10^{-7} mol/L. The manufacturer kindly supplied the compound. The ETA/ETB receptor antagonist LU 302 872 was added to the medium at the same time as administration of the various stresses were started. Confluent HPMCs were incubated in serum-free medium for 24 hours before starting the experiments. Cell viability was assessed by trypan blue. At the end of the experiments viability was more than 90%.

Analyzing osmolar effects

The cells were incubated in a serum-free medium supplemented or not with D-glucose or glycerol. The osmolytes were added to the medium to increase the osmolarity from 290 mOsm/kg (normal osmolarity) to either 300 mOsm/kg (low osmolarity condition) or 500 mOsm/kg (high osmolarity condition). Osmolarities were measured by the freezing point depression method with a micro-osmometer. For the measuring of the ET-1 concentration in the supernatant, conditioned media were collected every 24 hours for 96 hours.

Analyzing physical forces

Reports on fluid-induced shear stress are generally based on laminar flow models. However, in peritoneal dialysis fluid, fluctuation within the abdomen generates nonlaminar fluid shear stress. We attempted to mimic these effects. HPMCs were placed onto a rotating shaker (KS 125 Basic) (IKA Labortechnik, Staufen, Germany) and fluid shear stress was induced by a constant rotation of the plates (100 rpm). To analyze the effect of cellular stretch, we used a method similar to that described by Wirtz and Dobbs [18]. HPMCs (5×10^4 cells/25 mm diameter well) were seeded into silicone elastomer 6-wells plates (Flex I plates) (Flexercell, McKeesport, PA, USA) and cultured in 2 mL/well medium. The membranes were stretched by applying cyclic air pressure from below. Air pressure was pump-driven and valve-controlled. The frequency of oscillations was set at 50 cycles/min. A 20% increase in the membrane surface area was targeted. The surface area of the elastic membrane was determined by measuring the height of membrane displacement and calculating the increase in surface area from the formula for the area of a sphere. According to Wirtz and Dobbs [18], a 20% increase in the surface area of a silicone membrane translates in an effective increase of cellular surface area of approximately 15%.

Mesothelial cells were exposed to fluid shear stress or cellular stretch for 72 hours.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA from cultured HPMCs was extracted with Trizol (Life Technologies) and reverse transcribed into single-stranded wild-type cDNA (GeneAmp RNA PCR Core Kit) (Perkin-Elmer, Branchburg, NJ, USA) according to the manufacturer's instruction.

The single-stranded wild-type cDNA was subjected to a PCR with modified primer pairs in order to create internal cDNA competitive reference standards (CRS). All DNA sequences used for the design of the PCR primers were obtained from *www.ncbi.nlm.nih.gov*. The downstream primers were complementary to the mRNA strands. Their 3′ ends were linked to an additional sequence, complementary to a region 50 to 70 bp upstream on the mRNA in relation to their 5′ starting sequence. This resulted in a 50 to 70 bp deletion in the final PCR-product. The upstream primers were identical in sequence to the mRNA strands except that their 5′ ends were coupled with a T7-promotor sequence.

The PCR product was separated on a 2% agarose gel and stained with ethidium bromide. In order to ensure identical PCR conditions for the template and for the CRS, a single-stranded cDNA CRS was generated. Therefore, double-stranded cDNA bands were excised from the gel, eluted using the Quiagen Gel Extraction Kit® (Quiagen, Hilden, Germany) and transcribed into cRNA with the Riboprobe T7 System® (Promega, Madison, WI, USA). The amount of extracted cRNA was determined by ultraviolet spectrophotometry at 260nm, and 0.5μg cRNA were reverse transcribed into internal single-stranded cDNA CRS. Finally, the concentration of single-stranded cDNA was determined.

Table 1. Primer sequences for the construction of internal cDNA control reference standards, for competitive polymerase chain reaction (PCR) as well as for the characterization of the endothelin system [endothelin-1 (ET-1), ETA and ETB rezeptor, endothelin-converting enzyme]. Internal cDNA competitive reference standards (CRS) were constructed using modified primers. The 5′ end of the upstream-primer (sense, sequence 1) was coupled with a T7- promoter sequence. The 3′ end of the downstream-primer (antisense, sequence 1) was linked to an additional sequence (sequence 2), complementary to a region 50 to 70 bp upstream

		Primer Nucleotide-Sequence	First nucleotide of the primer sequence *bp*	Estimated PCR product size *bp*
Collagen I				
Sense	T7 promoter	5′-AAT TTA ATA CGA CTC ACT ATA GGG A-3′		
	Sequence 1	5′-CG CAC ATG CCG TGA CTT GAG ACT CA-3′	606	
Antisense	Sequence 1	5′-CCA CCG ATG TCC AAA GGT GCA AT-3′	1155	572
	Sequence 2	5′-A GCA GCC ATC TAC AAG AAC AGT GTA A-3′	1052	495
GAPDH				
Sense	T7 promoter	5′-AAT TTA ATA CGA CTC ACT ATA GGG A-3′		
	Sequence 1	5′-CG TCT TCA CCA CCA TGG AGA A-3′	375	
Antisense	Sequence 1	5′-CCA GTG AGC TTC CCG TTC AGC TC-3′	742	390
	Sequence2	5′-TCC GGG AAA CTG TGG CGT GAT GGC 3′	648	321
ET-1				
Sense		5′-GCA GTC TTA GGC GCT GAG CTC AGC G-3′	267	458
Antisense		5′-AGG TGT TCC TCT GAA CTT CTT CTG ATT-3′		
ETA rez				
Sense		5′-GCA GAA GTC CTC GGT GGG GAT CA-3′	976	570
Antisense		5′-CCG ATG TAA TCC ATG AGC AGT AAG-3′		
ETB rez				
Sense		5′-GGA CCC ATC GAG ATC AAG GAG ACT-3′	511	504
Antisense		5′-GGG ATG AAG CAA GCA GAT TCG CAG A-3′		
ECE				
Sense		5′-CCC GCC ATC AAC TGG TGG CCT TT-3′	1055	779
Antisense		5′-CAG CTC ATG GCC CAC GAC GAC AC-3′		

Abbreviations are: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ET-1, endothelin-1; ECE, endothelin-converting enzyme.
All DNA sequences used for the design of the PCR primers were obtained from *www.ncbi.nlm.nih.gov*. All PCR products were sequenced to confirm identity.

Constant amounts of single-stranded wild-type cDNA (0.5 μL) together with serial dilutions [1:3 for collagen I, 1:5 for glyceraldehyde-3-phophate dehydrogenase (GAPDH)] of single-stranded cDNA CRS (starting concentrations, 2 μg) were coamplified in a PCR mixture for which we used non-modified primer pairs (Table 1).

PCR products were then directed to agarose gel electrophoresis (2%), visualized by ethidium bromide staining, and photographed with a digital camera (Kodak DC 40, Rochester, NY, USA). Two different bands were distinguishable due to the smaller size of the cDNA CRS as compared to target cDNA. As charged by comparison with a 100 bp DNA-ladder (Rapidozym, Berlin, Germany), PCR products proved to be consistent with their predicted size. Sequencing of the PCR products confirmed the identity of the amplified target genes (Replicon, Berlin, Germany). Band intensities were densitometrically analyzed through the use of the One-Dimensional Image Analysis Software® (Kodak Digital Science™). The relative amount of target mRNA was calculated through the use of a log-log scale plot of the ratio of PCR products (cDNA CRS band intensities/target cDNA band intensities) versus the known amount of cDNA CRS used in the competitive PCR reactions. When target and CRS values were equivalent, the amount of target was equal to the known amount of internal CRS. In order to exclude nonspecific changes in total mRNA synthesis, the value of target mRNA was normalized to expression of GAPDH mRNA. Negative controls were performed with target cDNA only, with cDNA CRS only and without cDNA in order to exclude contaminating DNA.

Characterization of the mesothelial endothelin system by PCR

All DNA sequences used for the design of the PCR primers were obtained from *www.ncbi.nlm.nih.gov*.

Table 1 provides detailed information on primer sequences, the first nucleotide of the primer sequence as well as the estimated product size. All PCR products were sequenced to confirm identity.

STATISTICS

Statistical analysis was carried out with the SPSS System (Release 10.0). Only nonparametric tests were used for comparison. The Mann-Whitney *U* test was used for unpaired data, the Wilcoxon signed rank test was used for paired data. Results are expressed as mean ± SE. A P value <0.05 was regarded as significant.

RESULTS

Characterization of cultures of HPMC

Confluent cells were polygonal and formed a regular monolayer in light microscopy. They expressed cytokeratin and vimentin but failed to react with the endothelial marker von Willebrand factor.

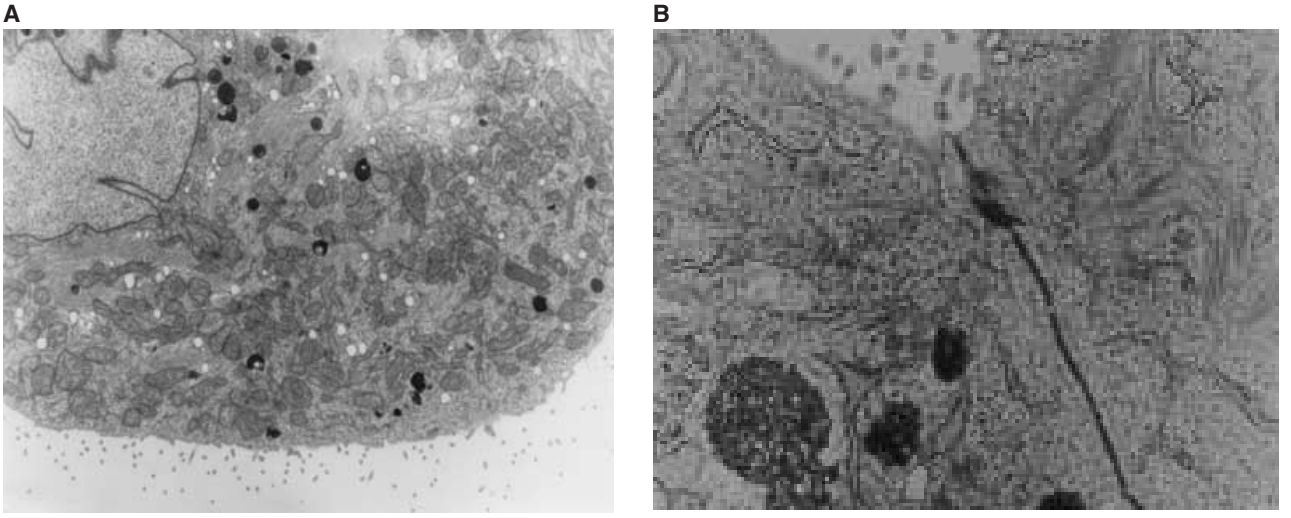

Fig. 1. Electron-microscopic examination of mesothelial cells. (*A*) Mesothelial cells express a huge number of microvilli on the surface. Micropinocytotic vesiscles can be observed on the cell membrane. In the cytoplasma, numerous organels can be detected such as the endoplasmic reticulum, lamellar bodies, mitochondria, and the Golgi complex. (*B*) Mesothelial cells are tightly bound to each other by cell interaction. In this scan a tight junction is shown.

By electron microscopy, mesothelial cells showed apical microvilli and tight junctions (Fig. 1).

Characterization of mesothelial endothelin system by PCR

Mesothelial cells expressed genes for ET-1, the ET-A and ET-B receptors, as well as for the endothelin-converting enzyme.

Osmotic agents and mesothelial ET-1 release

Confluent mesothelial cell cultures exposed to glucose media showed a marked increase in ET-1 release compared to glycerol or unchallenged control medium (Fig. 2). High glucose (500 mOsm/kg) medium led to the highest ET-1 release and values at 72 and 96 hours differed significantly from that of glycerol (500 mOsm/kg) challenged or osmolyte-free medium (290 mOsm/kg) ($P < 0.05$). Low glucose (300 mOsm/kg) also had a tendency toward increasing ET-1 values over time ($0.1 > P > 0.05$). The percent increase in ET-1 release for high glucose was greater than that for high glycerol medium compared to osmolyte-free medium. At 72 hours, mean values were 78% ± 18% for glucose and 5% ± 9% for glycerol ($P < 0.05$).

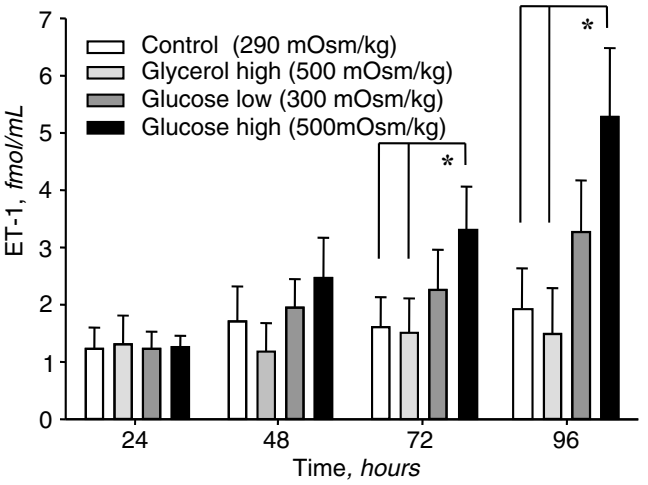

Fig. 2. Osmolar effects on human peritoneal mesangial cells (HPMC) endothelin-1 (ET-1) release. Glucose led to a concentration and time-dependent increase in mesothelial ET-1 release. Values at 72 and 96 hours differed significantly ($N = 10$ for each experiment). $^*P < 0.05$ from standard and glycerol medium.

Fluid shear stress

Fluid shear stress led to increased the mesothelial ET-1 release compared to nonstressed HPMCs. Mean ET-1 values at 72 hourw were 3.3 ± 0.4 fmol/mL for HPMCs exposed to continuous fluid shear stress and 2.1 ± 0.4 fmol/mL for nonstressed HPMCs ($P < 0.005$). Combining fluid shear stress with high glucose medium (500 mOsm/kg) did not increase ET-1 release further. Mean values were 3.0 ± 0.4 fmol/mL. Blocking the ETA and ETB receptors during the fluid shear stress experiments led to a significant increase in mesothelial ET-1 release ($P < 0.05$) (Fig. 3).

Cell stretch

Mesothelial cell cultures grown in standard medium and exposed to cyclic cellular stretch tended to show higher ET-1 values compared to nonstressed HPMCs ($0.1 > P > 0.05$). Mean values were 2.1 ± 0.2 fmol/mL for cellular stretch compared to 1.8 ± 0.4 fmol/mL for nonstressed cell cultures. Combining high glucose medium

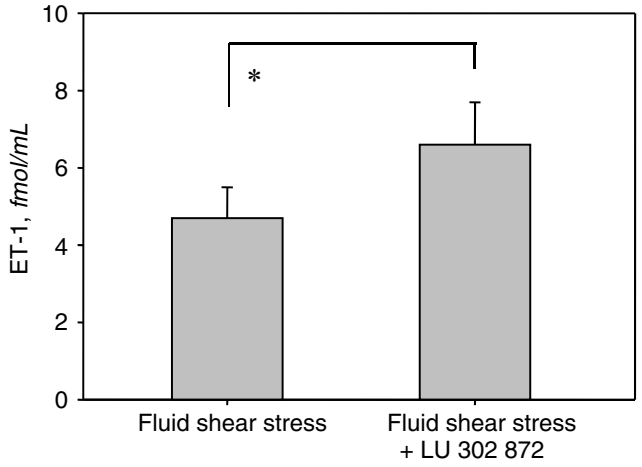

Fig. 3. Endothelin-1 (ET-1) release of human peritoneal mesangial cells (HPMC) exposed to continuous fluid shear stress (72 hours). Adding the combined ETA/ETB receptor antagonist LU 302872 to the culture medium led to a significant increase in ET-1 release ($N = 8$ for each experiment). $^*P < 0.05$.

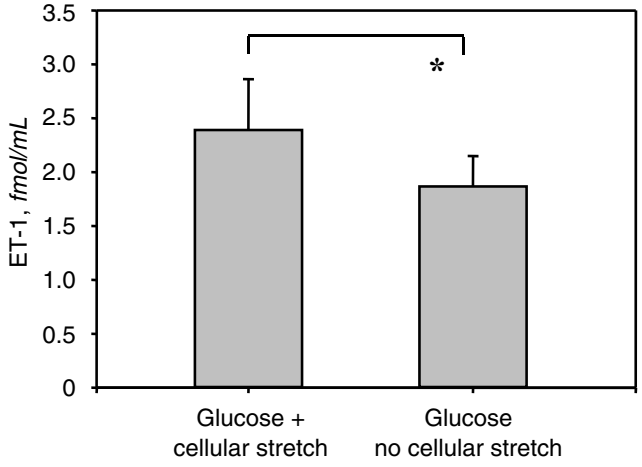

Fig. 4. Endothelin-1 (ET-1) release of human peritoneal mesangial cells (HPMC) exposed to high glucose medium (500 mOsm/L) with and without cellular stretch. High glucose medium in combination with cellular stretch led to a significantly higher ET-1 release compared to the unstretched controls ($P < 0.05$).

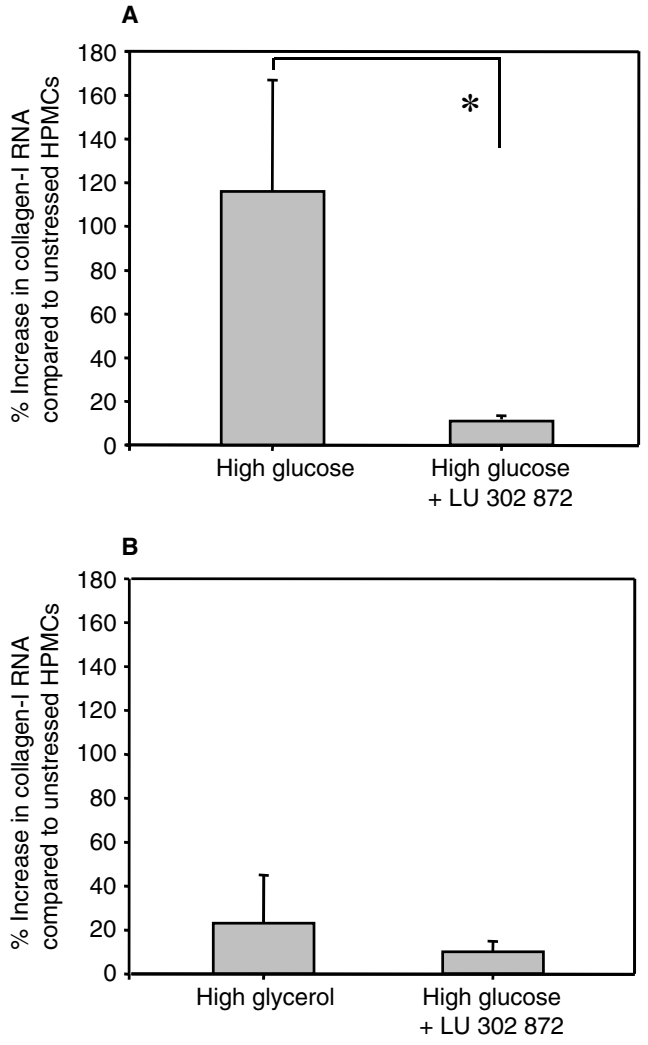

Fig. 5. Human peritoneal mesangial cells (HPMC) cultures. (*A*) HPMC cultured in high glucose medium (500 mOsm/kg) showed a significant increase in collagen I RNA synthesis compared to nonstressed controls. This effect was inhibited by the ETA/ETB antagonist LU 302 872. The percent increase in HPMC collagen I RNA release differed significantly between both groups (N = 3 to 8 for each experiment). $^*P < 0.05$. (*B*) Glycerol (500 mOsm/kg) had almost no effect on HPMC collagen I RNA synthesis ($N = 3$ to 8 for each experiment, NS).

and cellular stretch led to a significant increase in the mesothelial ET-1 release compared to nonstressed cells grown in high glucose medium ($P < 0.05$) (Fig. 4).

Collagen I RNA synthesis

HPMCs exposed to high glucose medium showed a mean increase of 118% (20% to 320%) in collagen I RNA synthesis compared to cells grown in standard medium ($P < 0.05$) (Fig. 5A). Glycerol led to a 23% (−49% to 75%) increase in collagen I RNA synthesis compared to unchallenged cells (NS) (Fig. 5B). Adding the combined ETA/ETB receptor antagonist LU 302 872 to mesothelial cell cultures incubated with high glucose media completely inhibited collagen I RNA synthesis (Fig. 5A). The combined ETA/ETB receptor antagonist had no effect in HPMC cultures grown in high glycerol or osmolyte-free medium (Fig. 5B). RT-PCR for collagen I RNA of HPMCs cultured in high glucose was greater than control medium (Fig. 6). Fluid shear stress increased collagen I RNA synthesis in HPMCs by 52% (19% to 110%) compared to nonstressed cells ($P < 0.05$). Adding the combined ETA/ETB receptor antagonist to cell cultures exposed to fluid shear stress reduced ($P < 0.05$) collagen I RNA synthesis to values of nonstressed HPMCs (Fig. 7). The combination of fluid shear stress and high glucose medium tended to achieve slightly higher values for

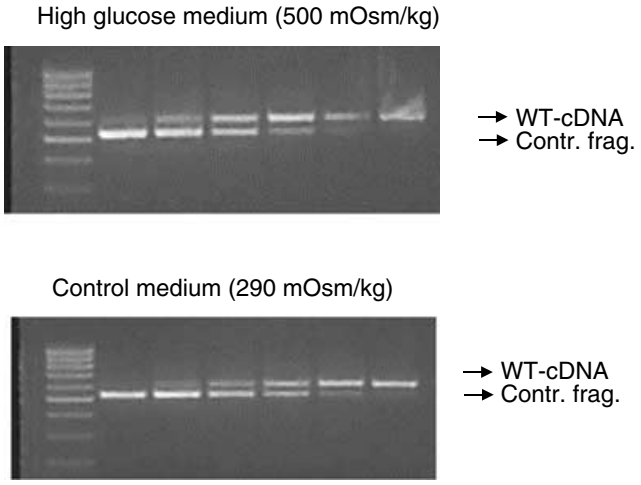

Fig. 6. Reverse transcription-polymerase chain reaction (RT-PCR) for collagen I RNA for human peritoneal mesangial cells (HPMC) cultured in high glucose (500 mOsm/kg) and control medium (290 mOsm/kg).

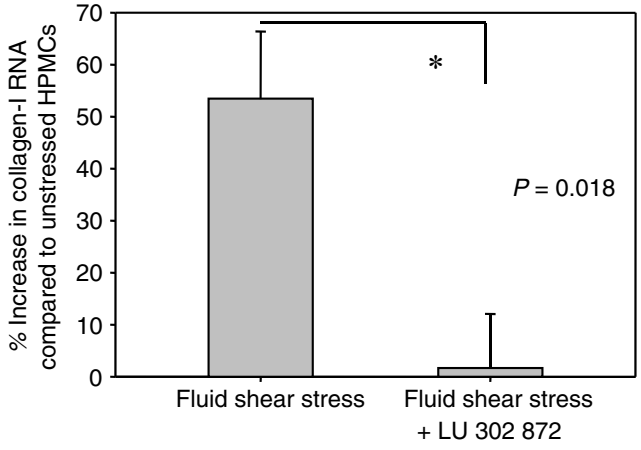

Fig. 7. Human peritoneal mesangial cells (HPMC) exposed to continuous fluid shear stress (72 hours) showed a significant increase in collagen I RNA synthesis compared to nonstressed controls. This effect was inhibited by the ETA/ETB receptor antagonist LU 302 872. The percent increase in HPMC collagen I RNA release differed significantly between both groups ($N = 8$ for each experiment). $^{*}P < 0.05$.

collagen I RNA synthesis ($0.1 > P > 0.05$). The effects of glycerol in combination with fluid shear stress were similar to those obtained with osmolyte free medium. Cellular stretch, either alone or in combination with glucose, had no significant impact on collagen I RNA synthesis.

DISCUSSION

We found that glucose increases HPMC ET-1 release in a time-dependent manner. These effects were observed at osmolarlities in the range of those of commercial dialysis fluids. The effects seemed to be glucose specific since high glycerol had no impact on ET-1. High glucose, but not glycerol, also increased HPMC collagen I RNA synthesis. The combined ETA/ETB receptor antagonist inhibited the increase. Fluid shear stress also increased HPMC ET-1 release and collagen I RNA synthesis. The ETA/ETB receptor antagonist inhibited the effect.

In good agreement with other studies [19–21], we could also demonstrate that high glucose concentrations induce synthesis of transforming growth factor-β (TGF-β) in HPMCs (data not shown). TGF-β is a well-known stimuli of endothelin [22]. Thus, the observed activation of the endothelin system might be, at least partially, secondary to the enhanced TGF-β release.

ET-1 has shown to directly stimulate collagen I synthesis in vascular smooth muscle cells from coronary arteries and the aorta [17]. Furthermore, ET-1 stimulates fibrogenic activity in various organs by regulating production and turnover of matrix components [23]. ET-1 is implicated in fibrotic diseases such as systemic sclerosis [24, 25], pulmonary fibrosis [26], and hepatic fibrosis [27]. Moreover, ET-1 overexpression in transgenic mice [16] and ET-2 in transgenic rats [28] causes renal fibrosis. These observations would support a possible mechanistic role of ET-1 in peritoneal fibrosis. We recently observed that by increasing the dwell volume or the glucose-induced osmolarity of the peritoneal dialysis fluid, we could activate the peritoneal paracrine endothelin system [15]. Our present findings suggest glucose and fluid shear stress are the primary triggers. In addition, we present evidence that links the activated mesothelial endothelin system to the profibrotic process within the peritoneum. Glucose is known as a potent stimulant for the paracrine ET-system [16, 29, 30]. Furthermore, high glucose concentrations may enhance ET-1 promoter activity in primary mesangial cells [31]. In experimental animal models of diabetes, blocking the ET-system has shown to ameliorate glucose-induced end organ damage [32–34].

The above-mentioned studies are especially important since the structural alterations of the peritoneum during peritoneal dialysis are very similar to vascular and tissue alteration seen in diabetes. The tissue alterations during long-term peritoneal dialysis include a thickening and replication of basement membrane of the peritoneal capillaries, angiogenesis as well as a thickening and fibrotic remodeling of the submesothelial tissue [35, 36]. Peritoneal dialysis has even been considered as an experimental model for diabetic microangiopathy in humans [37].

The exact mechanisms on how glucose induces fibrotic remodeling and basement membrane thickening are still under investigation. Evidence from in vitro studies of vascular cells suggest that an increased activation of protein kinase C (PKC) is intricately involved in this process [38, 39]. PKC activation has been associated with the activation of vasoactive factors such as vascular endothelial growth factor and ET-1 [40]. Hence, it is conceptually possible that some of the actions of PKC in hyperglycemia are mediated via ET-1. Chen et al [41] demonstrated

that endothelial cells, cultured in 25 mmol/L glucose, upregulated the expression of extracellular matrix proteins via ET-1 through activation of nuclear factor-kappa B (NF-κB) and activating protein-1 (AP-1). ET-receptor antagonism prevented extracellular matrix mRNA expression [34].

Also, vascular permeability seem to be effected by alteration of the endothelin system. This is of particular importance since vascular permeability directly impacts on transport characteristics of the peritoneal membrane during peritoneal dialysis [42]. Chen et al [43] investigated ET-1 expression in human umbilical vein endothelial cells (HUVEC) in response to glucose and the functional significance of these mechanisms. Permeability across HUVEC, grown in medium containing either low (5 mmol/l) or high (25 mmol/l) D-glucose were investigated. L-glucose was used as a control. Increased transendothelial permeability was noted in cells cultured in high glucose or when the cells grown in low (physiologic) glucose were incubated with ET-1 but not when they were incubated with L-glucose. Increased permeability was associated with increased ET-1, ETA, and ETB mRNA expression and augmented ET-1 immunoreactivity [43].

How fluid shear stress is converted into biochemical signals in the peritoneum is uncertain. Mechanical forces can affect the permeability of the cell membrane to various ions, such as Ca^{2+} or K^{+} [44, 45]. Mechanical stimulation can lead to cell depolarization by activating voltage sensitive channels [46]. Moreover, mechanosensitive ion channels have recently been identified [47] and characterized. Fluid shear stress had a strong impact on ET-1 release and collagen I RNA synthesis, while cellular stretch only had only a modest effect. Cellular stretch may represent a more physiologic stress for the intraabdominal mesothelial monolayer. Mesothelial cells are constantly exposed to cellular stretch during bowel movements or breathing which may have led to cellular stretch adaptation.

ETA/ETB receptor blockade was effective in blocking glucose-related and fluid shear stress–related effects in our study. ETA receptor and combined receptor antagonists have been effective in ameliorating ET-1–related effects in several animal models of fibrosis [48, 49]. In some of these studies, the receptor blockade was shown to involve reduced expression of growth factors, extracellular matrix deposition, and decreasing matrix metalloproteinase activity [50, 51].

We are aware that our study has limitations. Although glucose-related effects in peritoneal dialysis are easily appreciated, we have not shown for certain that fluid shear stress is a common phenomenon in peritoneal dialysis patients. Animal models of peritoneal dialysis should be the next step to address the impact of mechanical forces on the induction of peritoneal fibrosis more specifically. Nevertheless, we suggest the possibility that such stressors may be clinically relevant. We raise the hypothesis that high glucose combined with mechanical forces may stimulate collagen I RNA synthesis. Adding a combined ETA/ETB receptor antagonist that inhibited the effect. Although this approach appeared very effective, we did not examine other possible mediators that may nonethe-less be operative such as monocyte chemoattractant protein-1, fibronection, or advanced glycation end products. We believe our findings may have clinical relevance and application. If a locally applied ETA/ETB receptor antagonist were able to indeed ameliorate peritoneal injury in peritoneal dialysis patients, or possibly prolong the utility of the therapy, such a treatment would be of great value.

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